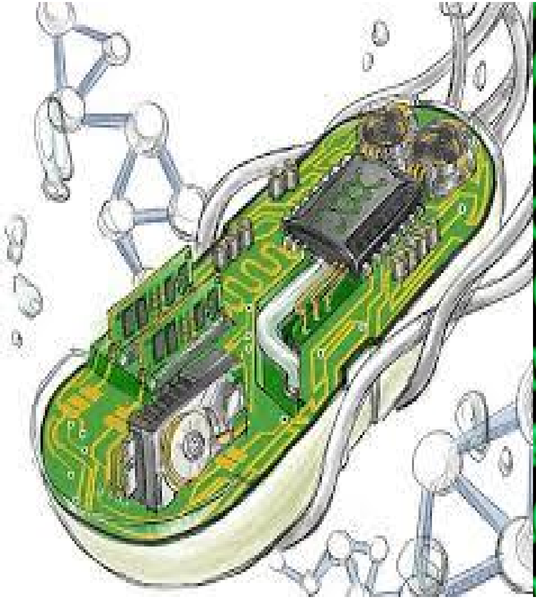
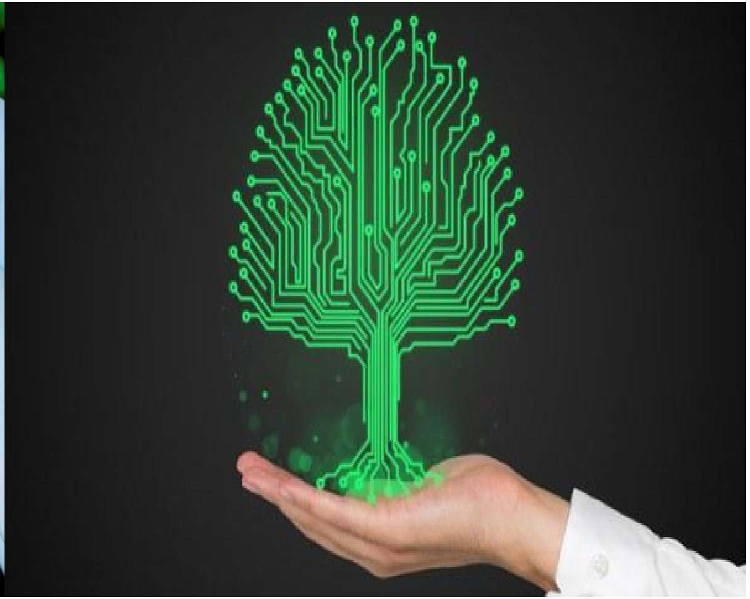
WELCOME TO THE 2016 IGEM SYNTHETIC BIOLOGY WORKSHOP

Synthetic Biology and the Future of Creation





Introduction

- **iGEM** is an international competition designed to encourage high schools and colleges to pursue solutions to problems worldwide through synthetic biology.
    - Each year, a convention is held to showcase each team's accomplishments and spread scientific knowledge.
- What is synthetic biology? There are two types:
    - The application of preexisting biological systems in ways other than their original use, almost as if we mimic the designs of natural structures
    - The design of new biological systems and parts

What else does iGEM do?

Apart from the collegiate and high school competition, iGEM also has two other purposes:

- **The Registry:** Through competitors and through independent labs, iGEM collects and stores biological parts available for open access to the entire synthetic biology community.
  - In synthetic biology, just like we use letters to make words, and words to make sentences, we use parts as our building blocks. We can combine parts to make different portions of a plasmid, which can be further combined to make a complete plasmid that performs a specific function. Plasmids will be discussed later.
- **Labs Program:** Labs doing independent research in areas such as universities are able to access the Parts Registry to conduct their own research and contribute to the field of synthetic biology.

Synthetic Biology Examples

Within iGEM:

- For the last two years, we have been developing an enzyme called chitinase that can break down chitin, a protein in the cell walls of many fungi and arthropods.

Outside of iGEM:

- Medical: The CRISPR/Cas9, a tool that can selectively modify certain parts of the genome, which is the collection of all human genes.
- Engineering: Various synthetic developments of spider silk, a material known for its strength. It has been synthetically produced and isolated in goat milk and from E. Coli.

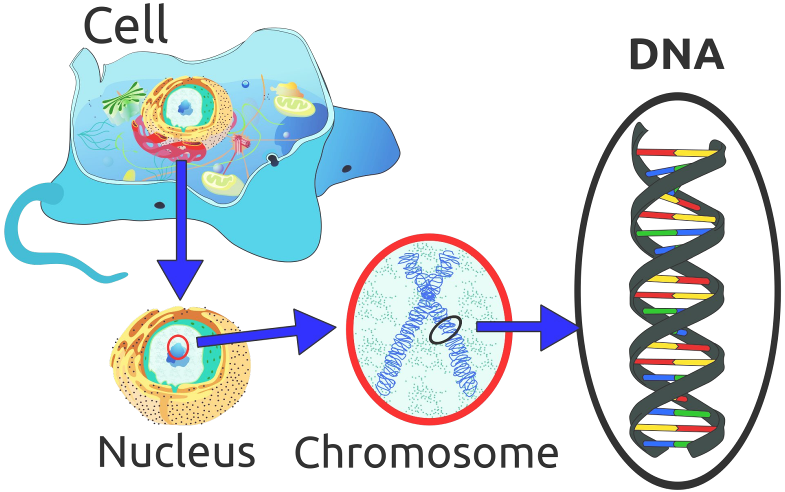
Spider Silk

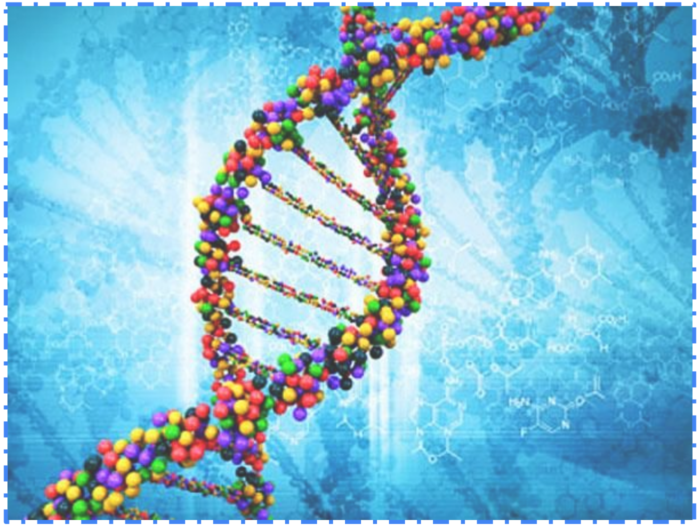
- Current research in spider silk involves its potential use as an incredibly strong and versatile material.
- Silk is completely biodegradable. If the production of spider silk ever becomes industrially viable, it could replace Kevlar and be used to make a diverse range of items such as:
    - Bullet-proof clothing
    - Wear-resistant lightweight clothing
    - Ropes, nets, seat-belts, parachutes
    - Rust-free panels
    - Biodegradable bottles
    - Bandages, surgical threads
    - Artificial tendons, ligaments or support for weak blood vessels

A dress made by silk from the Golden Orb Weaver Spider

What is DNA?

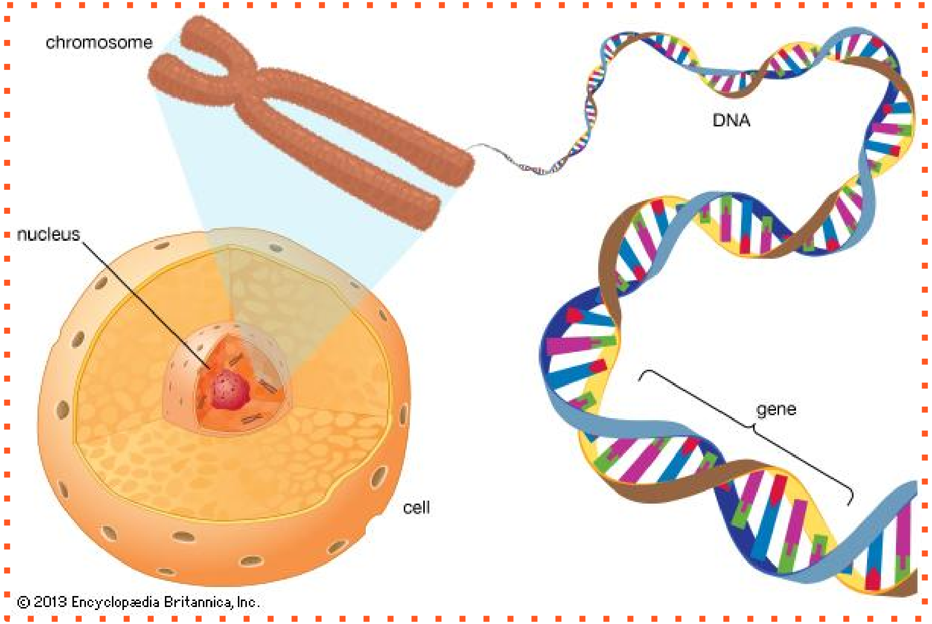
- DNA Stands for Deoxyribonucleic Acid
- It is present in ALL living organisms
- It carries the genetic information that makes you who you are
- Nearly every cell in a person's body has the same DNA





Where Can you Find DNA?

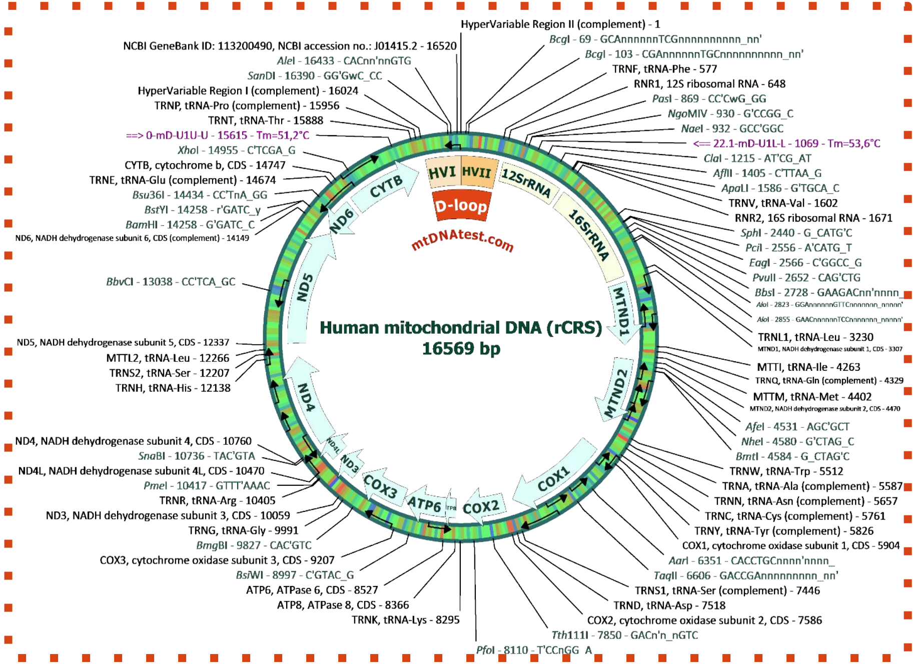
1. Most DNA is located in the cell nucleus



The DNA in the nucleus is divided between a set of different chromosomes. The human genome is distributed over 23 pairs of different chromosomes. Each chromosome consists of a single, enormously long linear DNA molecule associated with proteins that fold and pack the fine DNA thread into a more compact structure

Where Can you Find DNA?

1. Some DNA is also can also be found in the Mitochondria



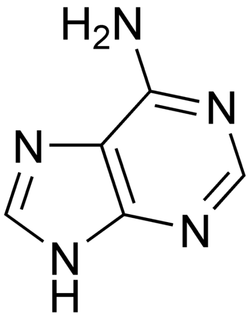
Mitochondrial DNA contains 37 genes, all of which are essential for normal mitochondrial function. Thirteen of these genes provide instructions for making enzymes involved in oxidative phosphorylation. The remaining genes provide instructions for making molecules called transfer RNAs (tRNAs) and ribosomal RNAs (rRNAs). These types of RNA help assemble protein building blocks (amino acids) into functioning proteins.

What is DNA Made Of?

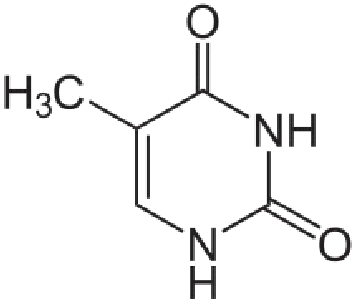
The information that DNA carries is stored as a code, so what is that code made of?

This code is made up of four chemical bases:

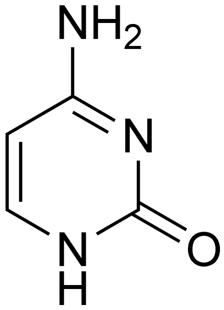
1. Adenine



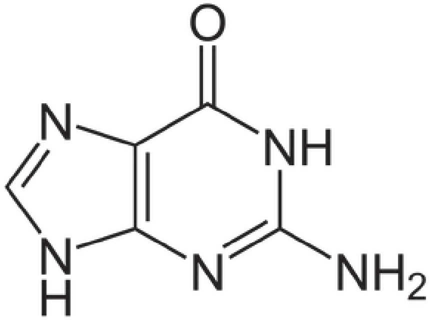
3. Thymine



2. Cytosine

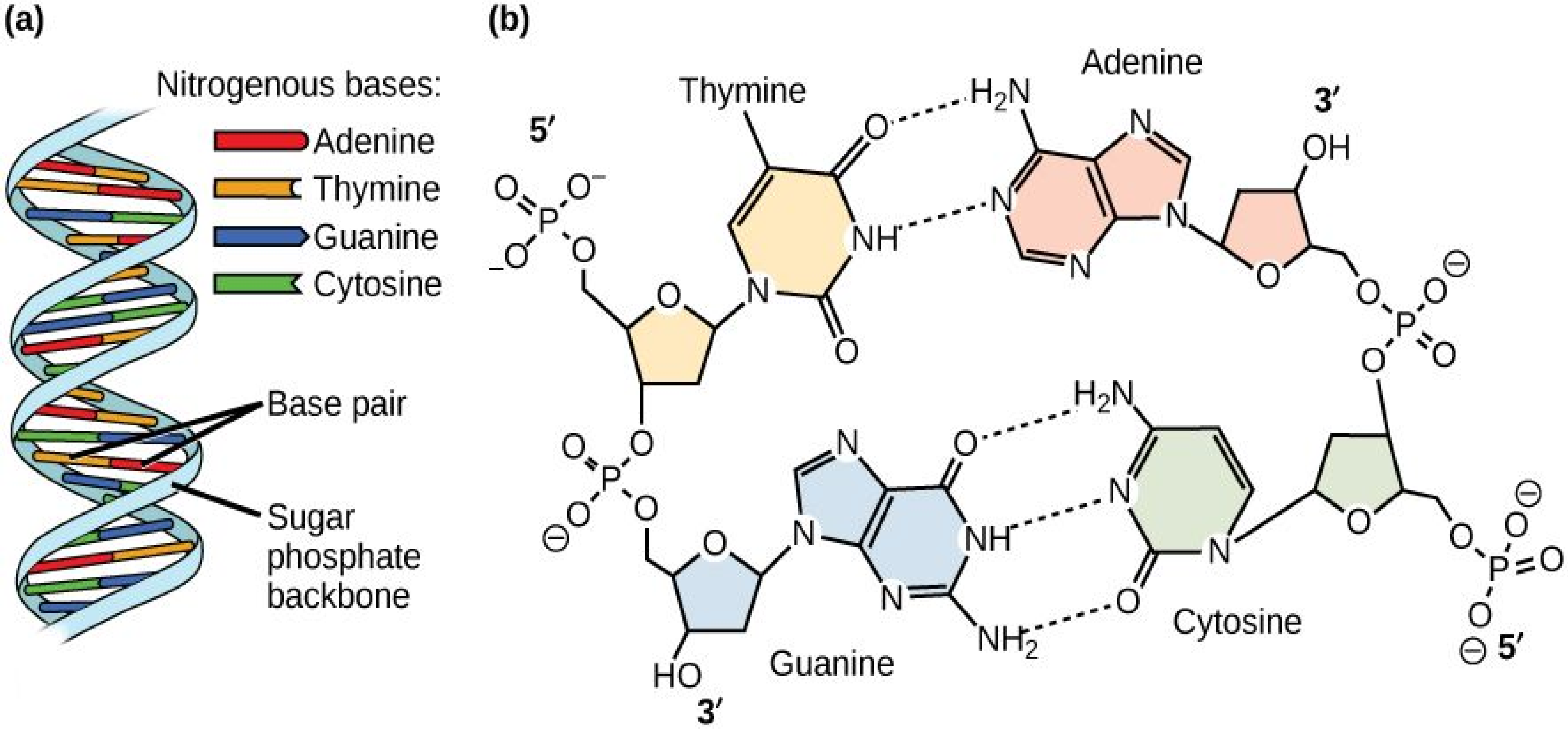


4. Guanine



What is the Structure of DNA?

- DNA bases have to pair with each other in a certain way
  - Adenine (A) pairs with Thymine (T) [double hydrogen bonds]
  - Guanine (G) pairs with Cytosine (C) [triple hydrogen bonds]
- Each base is also attached to a sugar molecule and a phosphate molecule
- a base + a sugar + a phosphate = one nucleotide
- The nucleotides are arranged in two long strands that form a spiral called a double helix
- This structure looks somewhat like a twisted ladder
  - The base pairs form the rungs of the ladder
  - The sugar and phosphate molecules form the vertical sidepieces of the ladder


(a)
Nitrogenous bases:
Adenine
Thymine
Guanine
Cytosine
Base pair
Sugar phosphate backbone
(b)
5′
Thymine
Adenine
3′
OH
H2N
NH
Guanine
Cytosine
HO
3′
NH2
5′

Fun Facts About DNA

1. If you could unwrap all the DNA you have in your cells, you could reach the moon 6,000 times
2. There are an estimated 3,000,000,000 DNA bases in our genome
3. It would take a person typing 60 words per minute, 8 hours a day about 50 years to type the entire human genome
4. In 2000, a rough draft of the human genome was completed and in 2003, the final draft was completed
5. Humans and chimps share anywhere between 94-99% of their DNA

DNA Pairing Race

Instructions:

1. You have been given one side of a DNA strand. Your job is to determine the corresponding sequence to complete the DNA.
2. Start when we say “GO!”
3. Winners get candy!!

How did you do?

Probably not as fast as the enzymes in your body.

Eukaryotic human DNA can replicate at an astonishing rate of 50 nucleotides per second!

DNA Replication

How Does Our Body Make Sure our DNA is Transferred From One Cell To Another During Cell Division?

DNA Replication has many steps. To Sum it up:

STEP 1: The human chromosome unwinds so that the double stranded helix can be in the proper conformation to separate.

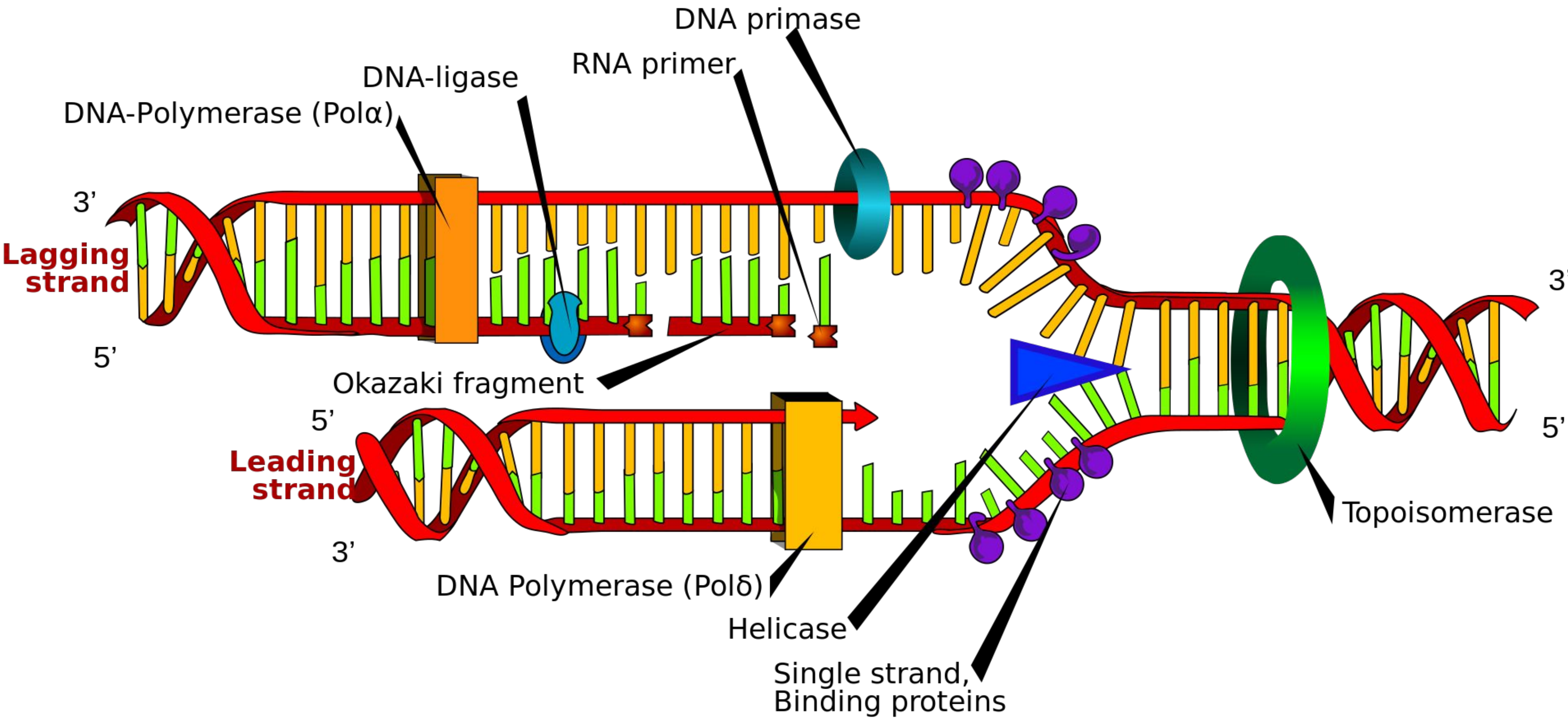
STEP 2: The double-stranded DNA helix separates/unzips, leaving two separate strands of complementary DNA.

STEP 3: The DNA Polymerase then comes and fills in the complementary base pairs.

STEP 4: The DNA Ligase then fills in all the gaps in the strands. The DNA ligase is important, because if it doesn't fill in all the gaps, mutations can occur which can result in cancer along with many other disorders.

STEP 5: The two strands of DNA then rewind back into the double helix structure allowing the cell the divide successfully.

DNA REPLICATION PROCESS



Animation:

https://www.youtube.com/watch?v=wcOZHK5bRLs

What is PCR?

1. PCR is sometimes referred to as “molecular photocopying”
2. Many labs use this technique to copy certain strands of DNA since it is fast and inexpensive.
3. The DNA is amplified through a series of heating and cooling processes that activate certain ingredients in the mastermix that help amplify the DNA.
4. The Mastermix acts as the ingredients for PCR. It contains primers,Taq DNA polymerase, buffers, DNTP mix, and magnesium chloride.

PCR Video

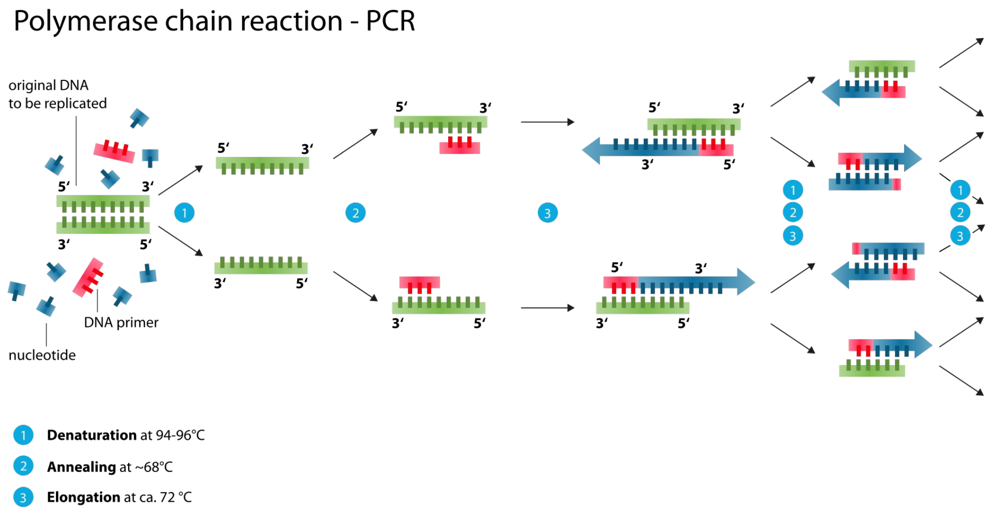
[PCR Video]

Summary:

PCR is the process in which a single piece of DNA is amplified through a series of heating and cooling processes. The ingredients for PCR are known as the PCR Mastermix. The mastermix includes a multitude of ingredients that help amplify a single strand of DNA. The mastermix, along with the DNA you wish to amplify are placed in multiple tubes that are then placed inside the PCR machine. Through a series of steps, the DNA is amplified using all of the ingredients in the mastermix. The DNA can then be used to identify certain trends and sequences that could be almost impossible to see if there was only one small copy.

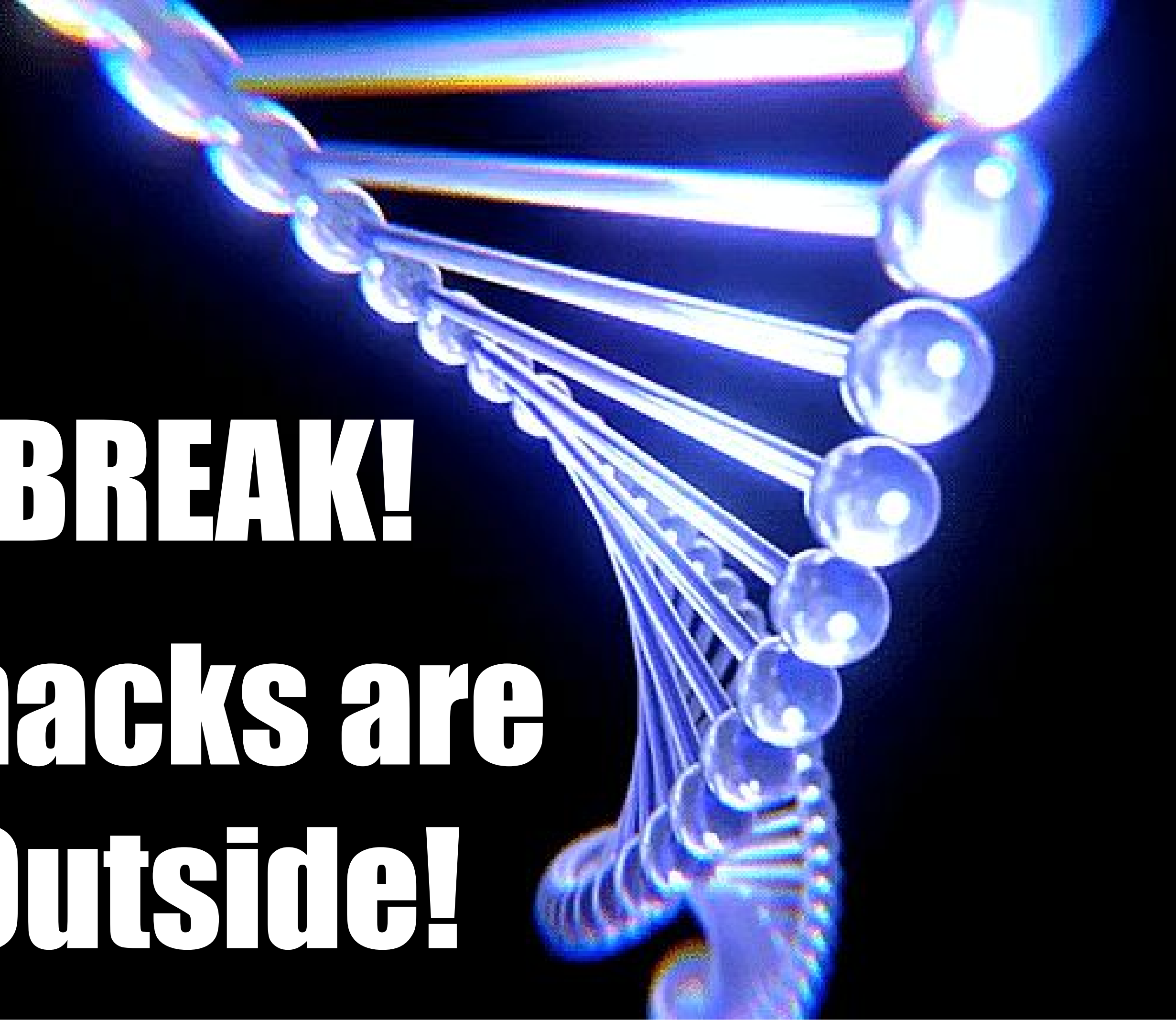
STEPS TO PCR

1. The DNA is heated so it denatures.
2. It then separates into two pieces, which are then used as templates.
3. This process results in the duplication of the original DNA.
4. The cycle of denaturing and synthesizing new DNA is repeated several times to make more than billion copies of the original DNA.



Applications of PCR to the real world: FORENSICS

PCR is used for a variety of different purposes in a multitude of fields. The one example I will be talking about, is forensics. Forensics is scientific tests or techniques used in connection with the detection of crime. So, for example, if a crime is committed inside of a house, the forensics officials would take DNA samples of all the objects that appear to be touched by the criminal. The DNA would then be put in a PCR machine, which would then provide the officials with multiple copies of the DNA. This would help the officials to determine whose DNA this was, allowing them to catch the criminal.



BREAK!
Snacks are Outside!

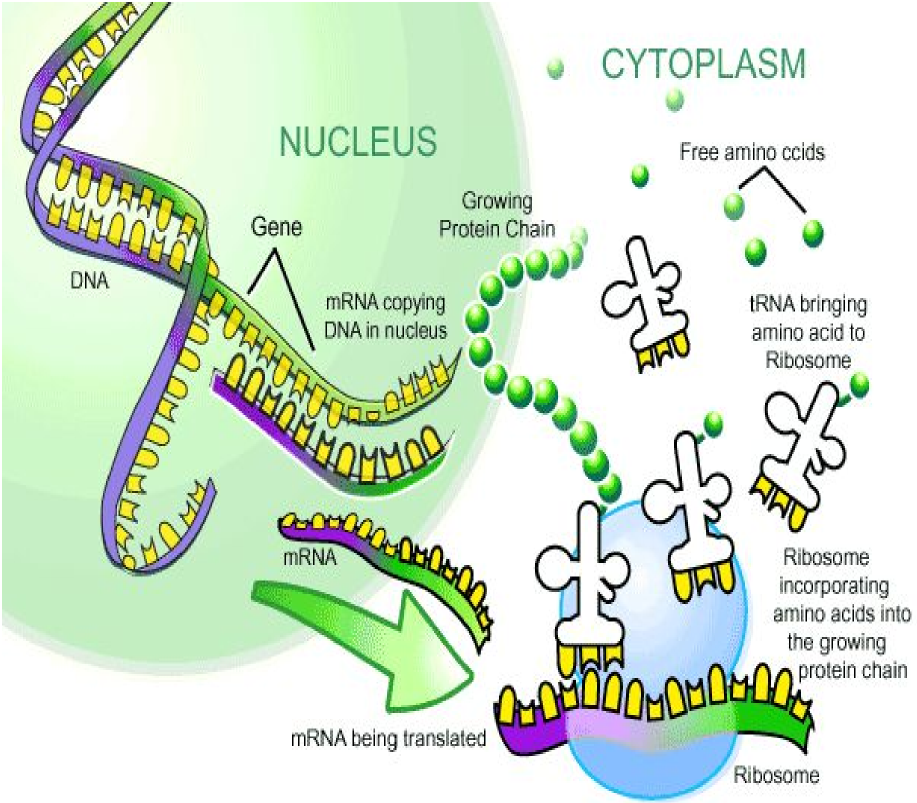
Introduction to Protein Synthesis

How does our body make proteins?

Why are proteins important?

- Proteins do a lot for our bodies! They help speed up bodily processes, replicate DNA, respond to our needs, and transport molecules from one place to another.

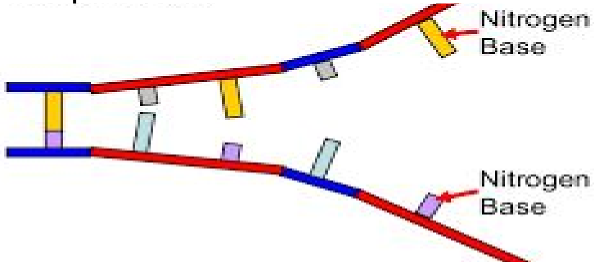
In order for the body to make proteins, the DNA must travel outside of the nucleus to the ribosome. This step is called ***transcription***. At the ribosome, a protein is formed from a chain of amino acids. This step is called ***translation***. Together, transcription and translation make up ***protein synthesis***.



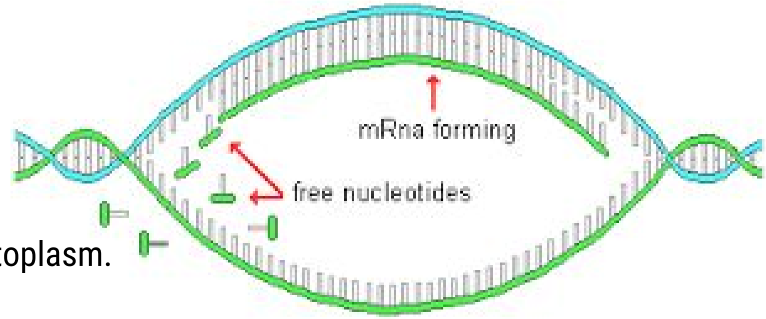
Transcription

The process of copying DNA to single stranded mRNA.

1. The DNA double helix untwists and unzips.



2. Free RNA nucleotides form base pairs with their matching nucleotides of DNA. (Uracil corresponds with adenine, taking the place of thymine.) The result is a single stranded RNA. This is called messenger RNA, or mRNA, because it carries the genetic "message" from the nucleus to the ribosome.

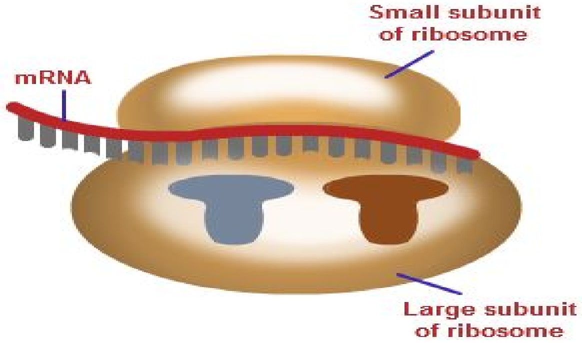


3. The mRNA leaves the nucleus to the cytoplasm.

Translation

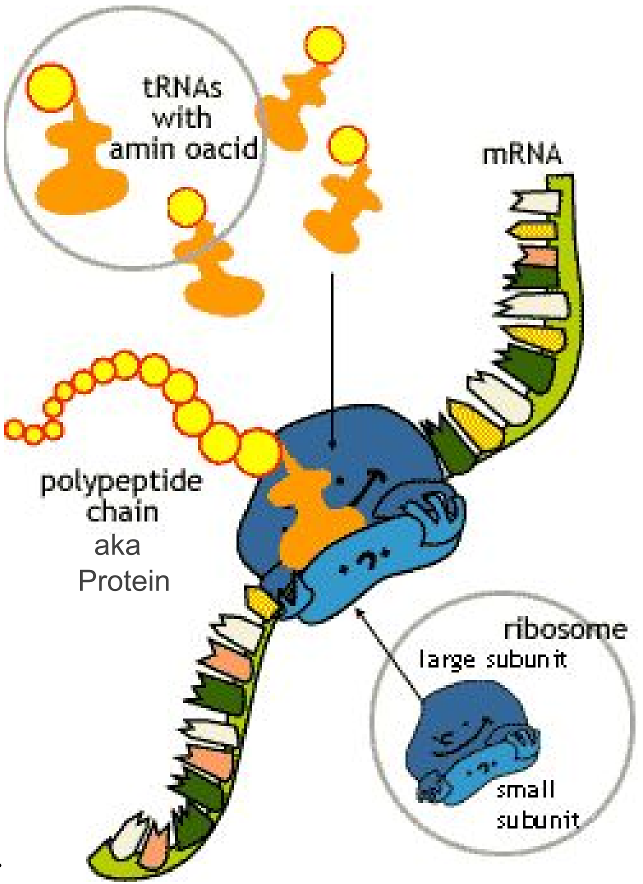
The process of using tRNA to create a chain of amino acids.

1. A ribosome attaches to the mRNA strand.



2. A tRNA anticodon matches with the mRNA codon. tRNA stands for transfer RNA. Each tRNA carries an amino acid and an anticodon, which is a 3-letter sequence that matches the mRNA.

3. The process repeats until a whole chain of amino acids form. This is a protein.



Protein Synthesis Exercise

Instructions:

1. Write down the corresponding mRNA sequence based on the given DNA sequence, in groups of three (codons). Remember that mRNA has Uracil (U) instead of Thymine (T).
2. Using the table, find the amino acid that matches the codon on the mRNA. A polypeptide chain usually starts with Methionine (Met) and ends with a Stop codon.
3. Write the first letter of each amino acid and find the secret message!

Example:

DNA:	TAC - AGG - TCC - CCG - GGT - AGT - CTG - AGA - TTC - ATC
mRNA:	AUG - UCC - AGG - GGC - CCA - UCA - GAC - UCU - AAG - UAG
Amino Acids:	Met - Ser - Arg - Gly - Pro - Ser - Asp - Ser - Lys - Stop

Sequence:

DNA:	TAC - CGT - CCC - TAA - ACG - GAT - TCT - TAT - AAG - ACA - TTA - TGA - TCA - ATT
mRNA:	
Amino Acids:	
Message:	___ ___ ___ ___ ___ ___ ___ ___ ___ ___ ___ ___ ___

Amino Acid Codes

Second Letter

1st letter	U		C		A		G		3rd letter
U	UUU	Phe	UCU		UAU	Tyr	UGU	Cys	U
	UUC		UCC	Ser	UAC		UGC		C
	UUA		UCA		UAA	Stop	UGA	Stop	A
	UUG	Leu	UCG		UAG	Stop	UGG	Trp	G
C	CUU		CCU		CAU	His	CGU		U
	CUC	Leu	CCC	Pro	CAC		CGC	Arg	C
	CUA		CCA		CAA	Gln	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU		ACU		AAU	Asn	AGU	Ser	U
	AUC	Ile	ACC	Thr	AAC		AGC		C
	AUA		ACA		AAA	Lys	AGA	Arg	A
	AUG	Met	ACG		AAG		AGG		G
G	GUU		GCU		GAU	Asp	GGU		U
	GUC	Val	GCC	Ala	GAC		GGC	Gly	C
	GUA		GCA		GAA	Glu	GGA		A
	GUG		GCG		GAG		GGG		G

ANSWERS

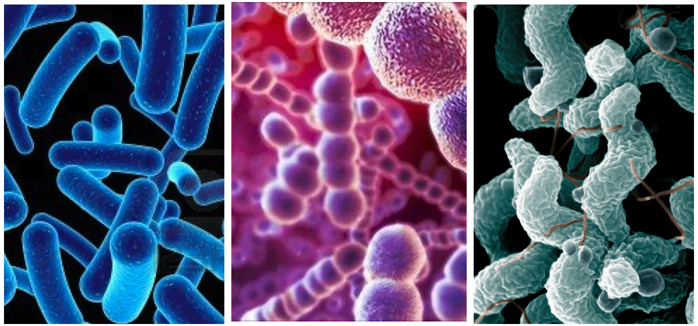
How did you do?!

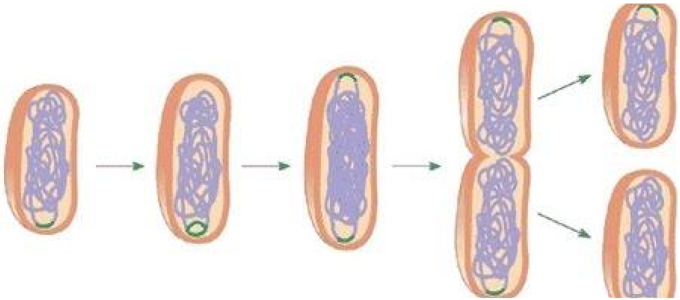
DNA:	TAC - CGT - CCC - TAA - ACG - GAT - TCT - TAT - AAG - ACA - TTA - TGA - TCA - ATT
mRNA:	AUG - GCA - GGG - AUU - UGC - CUA - AGA - AUA - UUC - UGU - AAU - ACU - AGU - UAA
Amino Acids:	Met - Ala - Gly - Ile - Cys - Leu - Arg - Met - Phe - Cys - Asn - Thr - Ser - Stop
Message:	M A G I C L A M P C A T S

Bacteria

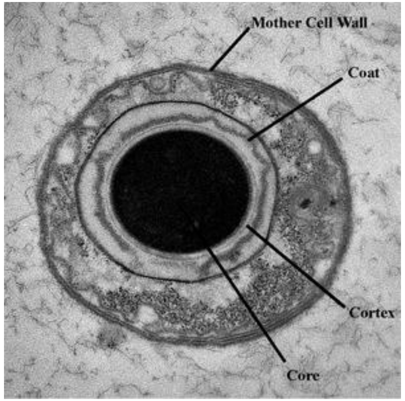
One of three domains in the taxonomy of living organisms

1. Prokaryotic: without a nucleus or nuclear membrane. Instead, its genetic code (DNA) is present as a long circular loop that is usually wound up in coils.
2. Single-celled organism: has a simpler cell structure than those in the domain Eukarya. For example, bacteria do not possess membrane-bound organelles (eg. mitochondria).
3. 3 Basic Shapes: cocci (spherical), bacilli (rod), and spiral.
4. Binary fission: the way bacteria reproduce. One bacterial cell replicates its DNA sequence and then splits into two, forming two identical "daughter" cells. DNA replication for bacteria usually starts at a specific sequence of base pairs, called the origin of replication.





Bacteria

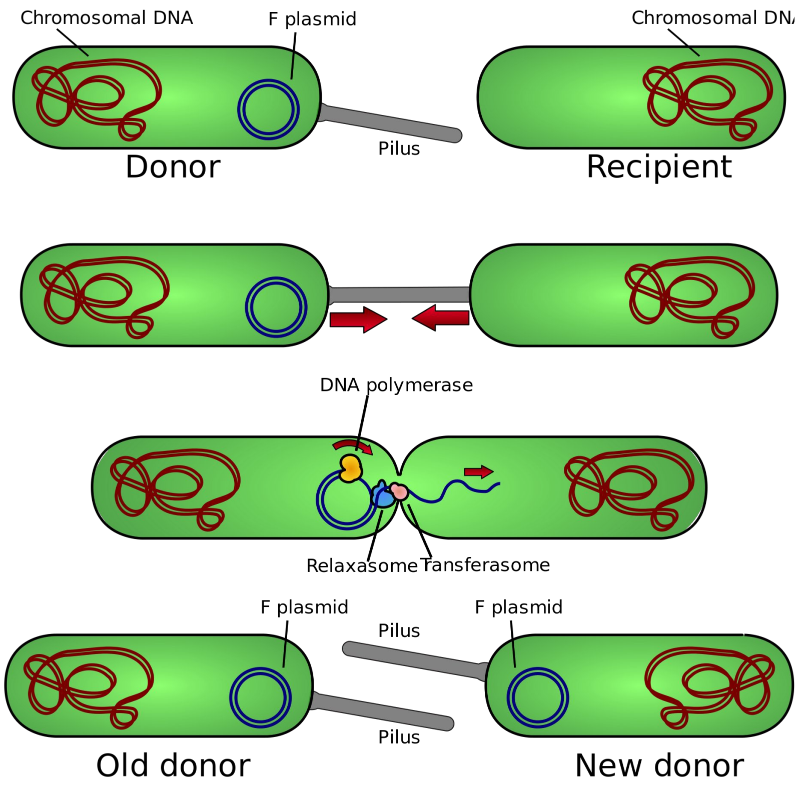


1. Where do bacteria live? Practically anywhere. They can thrive in extreme conditions such as boiling hydrothermal vents under the sea (such types of bacteria are called extremophiles). They are also present everywhere in daily life, in places like your gut (producing vitamins and gas).
2. What do they eat? Some bacteria are photosynthetic (need sunlight and nutrients) while others get energy from chemicals such as iron or sulfur. Certain bacteria also engulf smaller microbes to survive.
3. Cyanobacteria: This photosynthetic subclass of bacteria have existed since 3 billion years ago. It significantly contributed to the rising level of atmospheric oxygen back then.
4. Endospore: a structure formed by certain bacteria as a survival mechanism. It contains the bacteria's DNA and can survive extreme conditions such as high temperatures, UV radiation, and chemical damage.

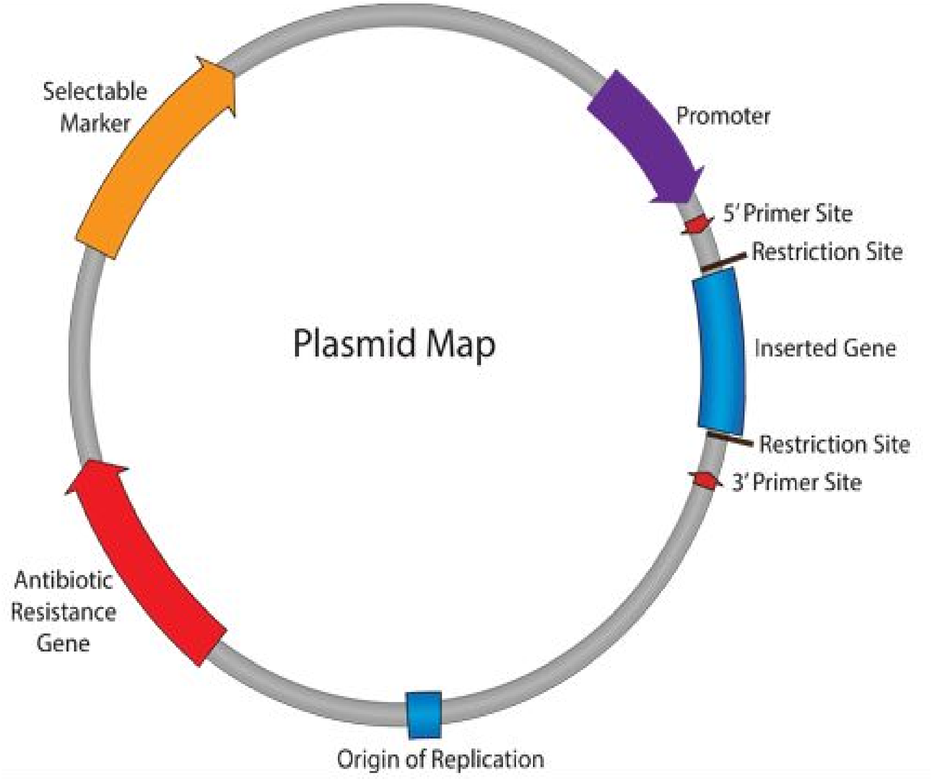
Plasmids

Mobile pieces of DNA present in bacteria

1. <u>Circular shape:</u> Each ring is double stranded and generally contains 1,000 to 20,000 base pairs.
2. <u>Purpose:</u> usually contains nonessential genes that give the bacterium a selective advantage. Most commonly, plasmids contain an antibiotic resistant gene that allows the bacterium to survive when it comes into contact with the specific antibiotic.
3. <u>Exchange:</u> Since plasmids are distinct from a bacterium's chromosomal DNA, they can be exchanged between bacteria. Bacteria can obtain plasmids from either conjugation (direct genetic material exchange through a physical connection between two bacteria cells) or intake from environment.



Plasmid Structure

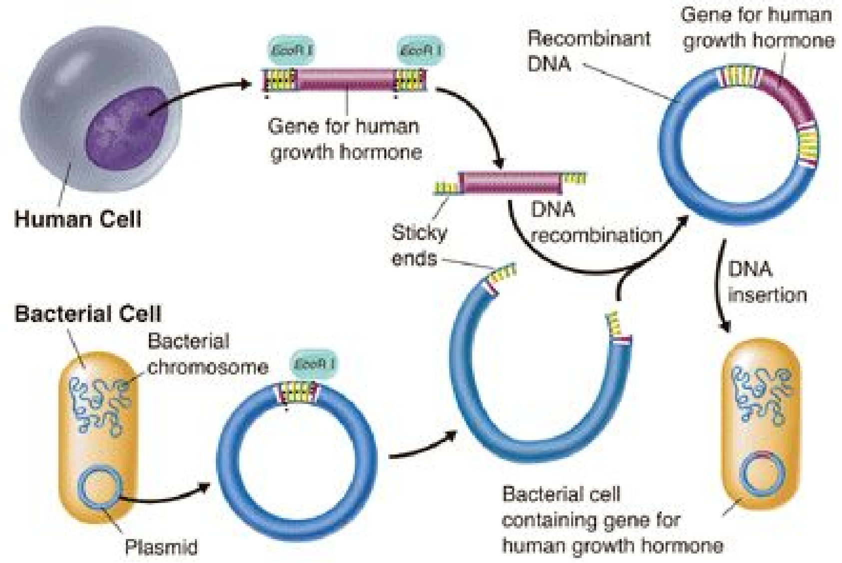


- Origin of replication: DNA sequence that is the starting point of replication for the plasmid.
- Antibiotic Resistance Gene: Allows scientists to select only the bacteria that contains the plasmid (a type of selectable marker).
- Promoter: Allows for the transcription of the target gene.
- Primer Sites: DNA sequences used in PCR amplification.
- Restriction Sites: Specific locations the restriction enzymes would cut in order to remove or insert a gene.
- Inserted Gene: The target gene that the scientists want the bacteria to express.

Plasmids in Synthetic Biology

Why are they so important?

- <u>Vectors:</u> Plasmids work as vectors, or delivery vehicles, to insert the desired foreign DNA into bacteria (the most common being E. Coli). The recombinant plasmid is an important step in the expansion of the biotech field, ever since it was first used in the 1970s.
- <u>Applications:</u> Bacteria could be manipulated to mass produce a variety of drugs and hormones, such as human insulin. The insulin produced would then serve its medical purpose.



Transformation Video

https://youtu.be/GNMJBMtKKWU

iGEM Project : Chitinase

For this year, our iGEM Project is focused on genetically engineering E. Coli to produce fungicide. By inserting a recombinant plasmid into bacteria, we can make the bacteria produce a type of enzymes called chitinases. The chitinases (strain LbCHI31 and LbCHI32) then break down chitin, a major structural component of fungi cell walls, therefore killing the fungi. A real life application of our project includes controlling fungi outbreaks among agricultural crops. Specifically, we target the fungi *Fusarium oxysporum*, which causes the Fusarium wilt in plants.

KAHOOT!

Go to Kahoot.it to play!